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## RESIDUAL CATALATIC ACTIVITY: A RAPID SCREENING TOOL TO VERIFY COOKING TEMPERATURE OF FISH AND SHELLFISH MEATS

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### Abstract

Inadequate cooking and improper holding times of processed foods are common causes of food-borne disease outbreaks, therefore, proper cooking is necessary to keep them safe for consumers. Residual catalytic activity test was employed to verify cooking adequacy of fish and shellfish meats between 60 and 90 °C with 5, 15 and 30 min holding times. The catalytic activity defined as the decomposition of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub>. The enzyme catalase retained its full activity up to 66 °C in both fish and shellfish meats. This sensitive test was able to detect cooking temperatures of fish and shellfish meats up to 74 and 72 °C respectively, which are the recommended safe temperatures (USDA-FSIS, 2001). The method provides a rapid and simple means for verifying adequacy of heat-processing of fish and shellfish meats.

*Key words:*. catalatic activity, cooking adequacy. Fish. shellfish

### INTRODUCTION

Heat treatment is given to fish and meat to produce a palatable product, to improve the shelf-life, and to minimize the risk of food-borne illness. However, inadequate cooking of food products and use of improper holding times are common causes of food-borne disease outbreaks ( Walton and McCarthy 1999; Uddin *et al.* 2000; Berry *et al.* 2001 ). To ensure consumer safety, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) recommended some minimum internal time/temperature heating combinations for specific food products (USDA-FSIS 2001). If products are overcooked, some food qualities could be lost. Consequently, optimum cooking is necessary to keep the maximum food qualities but minimize the risk of heat-labile pathogens. As most processed foods receive some type of heat treatment during

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processing prior to marketing or domestic cooking before consumption, a considerable number of studies have been carried out to develop methods for determining previous heat treatment of ready-to-eat meat products. Heat processing of marine foods has, however, received relatively little scientific study although it has an ever-growing role in human food source. We have conducting a series of study in this regard (Uddin 2001; Uddin *et al.* 2002a; Uddin *et al.* 2005 ).

A number of analytical methods have been developed to determine the previous heat treatment of processed meat and poultry products (Hsu *et al.* 2000). Among them, enzymes have been the locus of mans researches in relation to previous heat treatment of processed products primarily due to their characteristic loss of activity within a specific, narrow temperature range (Bogin *et al.* 1992; Uddin *et al.* 2002b). Spanier *et al.* (1990) suggested that catalase could be a good heating indicator of under-cooked beef since it considers a relatively thermostable enzyme in biological systems The catalytic activity defined as the decomposition of  $H_2O_2$  into  $H_2O$  and  $O_2$ . We have employed this simple and fist catalase activity test for predicting heating endpoint of fish and shellfish meats.

## **MATERIALS AND METHODS**

### **Fish and Shellfish**

Fresh red sea bream *Pagrus major* and kuruma prawn *Penaeus japonicus*, were purchased from local retail store and transported under refrigerated condition (4-5 °C) to the Laboratory of Food Processing, Tokyo University of Fisheries, Tokyo, Japan. Since most of raw materials used for the production of processed seafood are in the frozen state, the effect of freezing storage on the determination of catalytic activity was also evaluated. In this case fish and shellfish were kept at -40 °C for 2 months and removed from frozen storage then thawed overnight at 5 °C followed by catalytic activity determination.

### **Heat treatment**

Eighty gram each of meat was wrapped in polyvinylidene chloride film and immersed in a stirred water bath preheated to different temperatures between

60 and 90 °C. Temperature was monitored using a recorder (Thermodac EF, Model 5020A, Eto Denki Co., Tokyo, Japan) with connected thermocouples. Thermocouples (copper-constant) were inserted through the side edge of meat blocks to the geometric center. Samples were also heated for different holding times i.e. 5, 15, and 30 min. After reaching the specified temperature and holding time, the samples were immediately cooled in ice-cold water for rapid cooling followed by further analysis.

### CATALASE ACTIVITY

A portion of the geometric center of the sample was used for enzyme activity determination followed by the trimmed off outer layers of heated samples. The samples were chopped into fine pieces with a knife and 2 g aliquot were placed into the test tubes (2 cm diameter and 15 cm height) then 4 ml of 3 % H<sub>2</sub>O<sub>2</sub> were added. The contents of the tube were well mixed with a plastic stirring rod and incubated at room temperature (24 °C. Observations were made at 5, 10, 20, 30, and 60 min after addition of H<sub>2</sub>O<sub>2</sub>. Four determinations were carried out for each sample.

Based on the position of solid and liquid phases in the test tube, the catalytic activity of heated meat was classified as one of the following three types: (a) Positive the liquid layer was in the bottom of the test tube and all solids floated on the top. (b) Weak positive-liquid and solids were mixed or showed a slight separation or (c) Negative- the liquid layer was on top and all solids were in the bottom (Fig. 1 ).

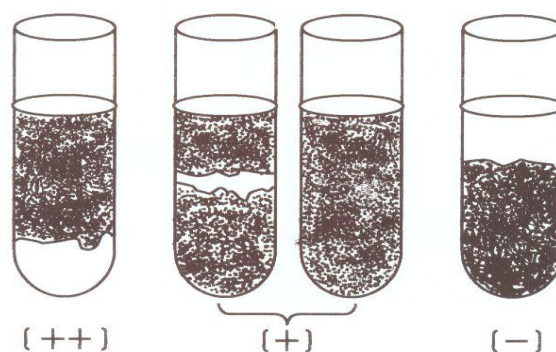


Figure 1- Catalatic activity test of cooked fish and shellfish meats.  
Reactions: positive ( ++ ); weak positive ( + ); negative ( - ).

## RESULTS AND DISCUSSION

Catalase is widely distributed in biological systems, reacting with  $H_2O_2$  to produce  $H_2O$  and  $O_2$ . The simplest procedure to test catalase activity is based on oxygen liberated upon reaction with  $H_2O_2$ . The results of the catalytic test heated fish and shellfish meats are presented in Table 1 and 2. Within 30 min the chemical reaction appeared to be stabilized therefore results of the 60 min incubation times are ignored. Initially the samples are heated 5 °C intervals between 60 and 90 °C (broad range) then the activity was monitored, however, the temperature range was finally reduced 2 °C intervals between 66 and 74 °C (narrow range) which is critical for food-labile pathogens in fish and fishery products (USDA-FSIS, 2001). All fish and shellfish meats heated up to 66 °C showed positive reaction even after 30 min holding times. During the determination of catalytic activity, foam or gas was generated when the cooked meat was mixed with 3%  $H_2O_2$  solution in a tube until a certain cooking temperature. Similar observations were also stated in heated beef and turkey breast meat (Spanier *et al*, 1990; Bogin *et al*, 1992).

Table 1. Catalytic activity of red sea bream meats in various cooking temperatures with different holding times

Temperature (oC)	Holding time (min)	Incubation time for catalase reaction (min)			
		5	10	20	30
66	5	++	++	++	++
	15	++	++	++	++
	30	++	++	++	++
68	5	++	++	++	++
	15	++	++	++	++
	30	+	++	++	++
70	5	++	++	++	++
	15	+	+	++	++
	30	+	+	++	++
72	5	+	+	+	++
	15	+	+	+	+
	30	-	-	+	+
74	5	-	-	-	+
	15	-	-	-	-

Reaction: positive (++); weak positive (+); negative (-)

Table 2. Catalytic activity of kuruma prawn meats in various cooking temperatures with different holding times

Temperature (oC)	Holding time (min)	Incubation time for catalase reaction (min)			
		5	10	20	30
66	5	++	++	++	++
	15	++	++	++	++
	30	+	++	++	++
68	5	++	++	++	++
	15	+	++	++	++
	30	+	+	++	++
70	5	+	+	+	+
	15	+	+	+	+
	30	+	+	+	+
72	5	-	-	+	+
	15	-	-	-	-

Reaction: positive (++); weak positive (+); negative (-)

In fish meat, catalase retained its full activity up to 68 °C and detectable activity was also noted at 70 °C with 30 min holding times. At 72 °C the weak-positive activity detected in the sample cooked for 15 min, however, the enzyme catalase completely lost its activity at 74 °C. This might be due to the insufficient concentration of undenatured catalase for detecting catalytic activity in cooked meats. Spanier *et al.* (1990) showed that 50 % of the catalase activity was retained after heating diluted, filtered beef homogenate at 74 °C. In this case the enzyme activity was determined by analyzing the concentration of H<sub>2</sub>O<sub>2</sub> retained in the reaction mixture rather than by observing the generation of foam or gas. One more possibilities in this case that beef catalase might more heat stable than those of fish and shellfish meats. In this study the mixture of cook meats and H<sub>2</sub>O<sub>2</sub> placed in a test tube and incubated for up to 60 min then observed the reactions (Fig. 1). When there was no enzymatic activity, all solids precipitated to the bottom of the test tube. But when there was residual activity, the gas/foam generated from the reaction raised the solid above the liquid phase even though no gas or bubbles are visible (seems weak positive reaction).

The results of catalytic reaction test for cooked shellfish meats were also showed similar patterns as fish meats, however, relatively lower detectable activity were noted (Table 2). Up to 70 °C with 30 min holding times., catalase retained its activity at weak positive level. Catalase lost its activity 100 % when

the meat was heated at 72 °C for 15 min holding times. It should be noted that there is an inverse relationship between catalytic activity and cooking temperature of meats. In a preliminary study we also observed the enzyme catalase lost its full activity at 72 °C with 30 min heating times in ezo-giant scallop meats. It is also revealed that cooking temperatures determined by the method employed in the present study were not significantly different between fresh/unfrozen and frozen samples, suggesting that the preliminary freezing procedure does not influence the catalytic activity test (data not shown). Therefore, it is very easy to demonstrate that there is no catalytic activity in a cooked fish or shellfish meat, the meat cooked over 72 °C. Since the factors such as: quality of products, kind/presences of additives, different cooking systems or heating rates which may affect the procedure have not been thoroughly investigated, these should be the subjects of further investigation.

According to the previous report, fully-cooked food products which are no need for further cooking except a brief warming, must be cooked up to an adequate temperature at least 71.1 °C (Townsend and Blankenship 1989; Hsu *et al.* 1999; USDA-FSIS 2001). The decreasing in catalytic activity as a function of increasing cooking temperature indicates the potential use of this test for estimating the maximum internal cooking temperatures of processed fish and shellfish meats. This residual catalase activity could be applied as a rapid screening tool to verify cooking temperature of fish and shellfish meats up to 72 °C.

## **CONCLUSION**

A sensitive catalytic activity test was developed for determining cooking temperatures up to 74 and 72 °C for processed fish and shellfish meats respectively. The method provides a rapid and simple means for verifying adequacy of heat-processing of fish and shellfish meats.

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